STUDENT NAME:

BIOL 2104 Take-Home Assignment: Print out these two pages (2 questions, each with multiple parts) and *write your answers in the spaces below*. This assignment is due by the beginning of class (8:35AM) on Monday, March 24th. The assignment is scored out of 10. Your assignment score will then be added to your Midterm 2 percent (%) score and this will be taken as your adjusted Midterm 2 mark (e.g. if you originally got 60% on Midterm 2 and score 10/10 on this assignment, then you get an adjusted final mark of 70% on Midterm 2).

Question 1 (worth 4 marks):

APOG is a hypothetical polymorphic short tandem repeat (STR) locus in humans with a repeating unit of **[CAGA]**. The polymorphic region is shown below as a box with 25 bp of flanking DNA sequence on each side. The *n* represents the number of times **[CAGA]** on the top strand is repeated (the polymorphic part of STR locus).

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5′-CTGATTCGTGATCTCCTTTAGCTTC[CAGA]  GTACCATTCGTTACGTGATAATGCC-3′
3′-GACTAAGCACTAGAGGAAATCGAAG[GTCT]n CATGGTAAGCAATGCACTATTACGG-5′
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(a) You plan to use PCR to genotype individuals for the *APOG* locus. If PCR primers must be 18 nucleotides long, design a pair of primers to amplify the *APOG* locus? Design the primers such that they are immediately adjacent to the [CAGA] repeat (i.e. don't worry about optimizing annealing temperature, GC content, etc. – everybody's primer sequences should be the same).

(b) Consider *APOG* alleles with 9 and 7 copies of the repeating **[CAGA]** unit. Using the primers you have designed, what will be the sizes in base pairs of the PCR products for each allele? In other words, if run on an electrophoresis gel, what would be the sizes of the two bands?

(c) There are five known alleles of *APOG* with 14, 11, 10, 9 and 7 copies of the repeating unit. How many possible human genotypes are there for these alleles, and what are they?

(d) If one parent is heterozygous for the 14 and 9 alleles of the *APOG* locus and the other parent is heterozygous for the 9 and 7 alleles, what are the possible genotypes of their offspring for this locus, and in what proportion will they be found?

Question 2 (worth 6 marks):

A double-stranded fragment of viral DNA, one of whose strands is shown below, encodes two polypeptides called *vir-1* and *vir-2*. Adding this double stranded DNA fragment to an *in vitro* transcription and translation system yields peptides of 10 residues (*vir-1*) and 5 residues (*vir-2*).

5'-AGATCGGATGCTCAACTATATGTGATTAACAGAGCCTGCGGCATAAACT-3'

(a) What are the nucleotide sequences of the open reading frames (start to stop codons, inclusively) within the mRNA transcripts coding for *vir-1* and *vir-2*? Write them out and designate the 5' and 3'-ends.

(b) What is the amino acid sequence of <u>each</u> polypeptide? Use one letter symbols to denote amino acids.

(c) In a mutant viral strain, the T at position 23 (counting from the left) on the DNA strand shown above has been replaced with G. Determine the amino acid sequences of the two peptides encoded by the mutant virus.

(d) What would be the sequences of the oligonucleotide primers (each 10 bp in length) required to amplify by polymerase chain reaction the DNA open reading frame (start to stop codons, inclusively) corresponding to the *vir-1* polypeptide? Be sure to designate the 5' and 3' ends of the primers. HINT: you only want the *vir-1* open reading frame amplified, not any flanking sequence.

(e) Draw the patterns of bands you would expect to see on the DNA sequencing gel below if you annealed the 10 residue primer 5'-CATATAGTTG-3' to the above single-stranded wild-type DNA template and carried out a dideoxy sequencing experiment (Sanger sequencing). The primer contains a radioactive label at its 5'-end.